AD NUMBER

ADB248915

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies only; Proprietary Info; Oct 98. Other requests shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, MD 21702-5012.

AUTHORITY

USAMRMC, ltr, 1 Jun 2001.

AD_____

GRANT NUMBER DAMD17-94-J-4191

TITLE: Transgenic Rat Models for Breast Cancer Research

PRINCIPAL INVESTIGATOR: Anne E. Griep, Ph.D.

CONTRACTING ORGANIZATION: University of Wisconsin System
Madison, Wisconsin 53706

REPORT DATE: October 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

NOTICE

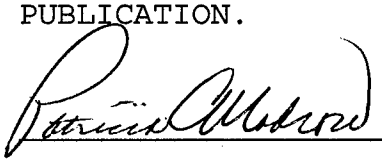
USING GOVERNMENT DRAWINGS, SPECIFICATIONS, OR OTHER DATA INCLUDED IN THIS DOCUMENT FOR ANY PURPOSE OTHER THAN GOVERNMENT PROCUREMENT DOES NOT IN ANY WAY OBLIGATE THE U.S. GOVERNMENT. THE FACT THAT THE GOVERNMENT FORMULATED OR SUPPLIED THE DRAWINGS, SPECIFICATIONS, OR OTHER DATA DOES NOT LICENSE THE HOLDER OR ANY OTHER PERSON OR CORPORATION; OR CONVEY ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE, OR SELL ANY PATENTED INVENTION THAT MAY RELATE TO THEM.

LIMITED RIGHTS LEGEND

Award Number: DAMD17-94-J-4191
Organization: University of Wisconsin System
Location of Limited Rights Data (Pages):

Those portions of the technical data contained in this report marked as limited rights data shall not, without the written permission of the above contractor, be (a) released or disclosed outside the government, (b) used by the Government for manufacture or, in the case of computer software documentation, for preparing the same or similar computer software, or (c) used by a party other than the Government, except that the Government may release or disclose technical data to persons outside the Government, or permit the use of technical data by such persons, if (i) such release, disclosure, or use is necessary for emergency repair or overhaul or (ii) is a release or disclosure of technical data (other than detailed manufacturing or process data) to, or use of such data by, a foreign government that is in the interest of the Government and is required for evaluational or informational purposes, provided in either case that such release, disclosure or use is made subject to a prohibition that the person to whom the data is released or disclosed may not further use, release or disclose such data, and the contractor or subcontractor or subcontractor asserting the restriction is notified of such release, disclosure or use. This legend, together with the indications of the portions of this data which are subject to such limitations, shall be included on any reproduction hereof which includes any part of the portions subject to such limitations.

THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.



10/6/99

_____ _____

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1998	Annual (1 Oct 97 - 30 Sep 98)

4. TITLE AND SUBTITLE
Transgenic Rat Models for Breast Cancer Research

5. FUNDING NUMBERS
DAMD17-94-J-4191

6. AUTHOR(S)
Anne E. Griep, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Wisconsin System
Madison, Wisconsin 53706

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, MD 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution authorized to U.S. Government agencies only (proprietary information, Oct 98). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

The laboratory rat is an important model for studying breast cancer due to the similarities in this disease between rats and humans. However, limited knowledge in manipulating the rat genome through transgenesis has prevented researchers from answering important questions in breast cancer research. We proposed to carry out detailed studies to optimize the variables in transgenic manipulation, to extend transgenic rat technology to inbred rat strains, and to develop rat embryo cryopreservation. We have evaluated multiple variables in the microinjection procedure and generated multiple transgenic rat lines which should be important for breast cancer research. We have developed an efficient cryopreservation procedure for rat embryos. Embryos from the outbred Sprague-Dawley and inbred Wistar-Furth and Copenhagen strains that possess unique characteristics for breast cancer research and multiple lines of transgenic rats that are useful for breast cancer research have been frozen. Lastly, due to the lack of rat embryonic stem cells that contribute to the germline, we have set up the technology for rat cloning, a technique which could allow researchers to generate germline mutations in cellular genes of the rat. This technology is critical for generating recessive mutations which are commonly described as the genetic basis for cancers.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Animal Models, Transgenic, Rats, Neu Oncogene, Embryo Cryopreservation, Breast Cancer	16
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

X In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

NA For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

X In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

X In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

NA In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Daniel Gus 12/22/98

PI - Signature Date

TABLE OF CONTENTS

Please note: Page 2 (abstract), Pages 8-13 contain unpublished data and therefore should not be distributed.

5. Introduction

5a. General Background

Breast cancer is one of the leading causes of death among women, with one out of every nine women in the United States being predicted to develop this disease during her lifetime. As with all cancers, breast cancer is a disease in which numerous cellular and molecular genetic changes are thought to contribute to the multistaged progression of normal cells to a population of cells with unrestricted growth and metastatic potential. Over the last decade two classes of genes, cellular protooncogenes and tumor suppressor genes, have been identified as genes which play critical roles in regulating cell growth and differentiation. Deregulation of gene expression through chromosomal translocation or mutation in the regulatory elements of the gene, alterations in the activities of these gene products through mutation in the coding regions of the genes, or complete loss of these genes from the chromosome through mutation are considered to be mechanisms contributing to the failure of cells to maintain normal growth characteristics.

Both mice and rats have been extensively used as laboratory animal models in breast cancer research, as well as in cancer research in general. For several reasons, the rat is perhaps the more suitable of the two with respect to a model system for human breast cancer. Whereas a high percentage of breast cancer in the mouse is associated with the integration of the mouse mammary tumor virus (MMTV) into the *int*-1 locus with consequent deregulation of *int*-1 expression, there is no known viral etiology of breast cancer in rats, as in humans (1). Second, the progressive disease that leads to breast cancer in laboratory rats bears striking histological similarity to that seen in human breast cancer (2-4). Third, a high percentage of the resulting rat mammary cancers are hormonally responsive, closely mimicking that seen in human breast cancer. Finally, certain inbred strains of rats show susceptibility to breast cancer whereas others show resistance (5,6). Through genetic crosses between these strains, putative suppressors have been identified (7-9). This genetic susceptibility to breast cancer seen in the rat may bear similarity to the human disease where genetic predisposition is considered to be an important factor (10,11). In part for these reasons, the rat is accepted as the animal model of choice for screening chemopreventive drugs for human breast cancer therapy (2).

Transgenic mice have been widely used in breast cancer research. Mouse models have been developed in which the expression of deregulated *int*-1 (12), c-*myc* (13-16), activated H-*ras* (13, 17), activated c-*neu* (18-20), wild type c-*neu* (21), deregulated growth hormone (22), and deregulated transforming growth factor a (23-25) has occurred in mammary tissue. All cases lead to abnormalities in mammary epithelial cells ranging from epithelial cell hyperproliferation without tumor formation to tumor formation, apparently some being similar to ductal carcinoma in situ which is seen in human breast cancers. The most prevalent genetic alterations in human breast cancers appear to be amplification of the c-*neu* locus (26-28), found in approximately 20% of breast cancers, and mutations of p53 (10,11). Unfortunately, discrepancies between the phenotypes of the several activated-*neu* transgenic mouse models has resulted in the lack of a consensus as to the nature of the activities of the *neu* oncogene in mammary carcinoma in these models. A more promising result was obtained from investigators who analyzed transgenic mice with deregulated expression of the wild-type *neu* proto-oncogene in mammary tissue. These mice developed focal mammary carcinomas, but only after long latency. The loss of p53 function through gene knockout led to only a very low percentage of animals with mammary adenocarcinoma (1 out of 26 p53 null mice) whereas there was a high incidence of malignant lymphomas (20 out of 26). These studies provide the best animal models to date for studying the correlation between disruptions in expression or activities of these cellular

genes and the incidence of mammary carcinoma. However, they may not be truly reflective of the genetics, histopathology, or the progressive nature of human breast cancer.

Considering the depth of knowledge generated by previous studies of breast cancer in the rat and the striking parallels between the rat and human disease, the availability of transgenic rat technology would greatly enhance breast cancer research. Transgenic rats would provide an alternative, and perhaps more suitable, animal model for dissecting the molecular mechanisms of mammary carcinogenesis and testing putative therapeutic agents. In addition to providing good models for breast cancer, the rat has been widely used for biochemical and metabolic studies, owing to its larger size. A large portion of research in neuroanatomy and neurophysiology is based upon the rat. The rat is the animal in which the multistage nature of hepatocarcinogenesis has been established and studied (29). All these areas of research would profit immensely from the availability of transgenic rats.

Recently, the Transgenic Animal Facility at the University of Wisconsin Biotechnology Center developed the capacity to generate transgenic rats, primarily with the encouragement of two university colleagues, Dr. Henry Pitot, an expert in hepatocarcinogenesis, and Dr. Michael Gould, an expert in breast cancer. Through our initial attempts at transgenic rat production, we have successfully generated transgenic rats for each of these cancer researchers. However, the state of transgenic rat technology is rudimentary compared to that for transgenic mice and as such has received only limited use to date. Despite our initial successes, the production of transgenic rats is at present an extremely laborious task. As a consequence of the technical impediments we now encounter, the time and cost for generating transgenic rats is many fold higher than that for the generation of transgenic mice. For many investigators, this high cost is prohibitive. Thus, only with further improvements will this technology be as accessible for the generation of transgenic rats as it has been for the generation of transgenic mice.

Because we foresee a long term and expanding demand for transgenic rats, especially in the breast cancer research field, **we proposed an investigation designed to** optimize transgenic rat production. This proposal to optimize transgenic rat technology was initiated because we believe that significant improvements can be made in both microinjection and embryo transfer techniques which would greatly facilitate transgenic rat technology. These advances should lead to the reduced cost in the production of transgenic rats, and to the capacity to generate transgenic rats in inbred backgrounds. Importantly, during the course of our optimization studies, a series of transgenic rat models for breast cancer research will be generated.

5b. Specific Aims and Statement of Work

Therefore, we proposed this infrastructure enhancement grant to provide a resource to the breast cancer research community for the generation of novel transgenic rat models for breast cancer research. The specific aims we proposed are:

(1) To generate transgenic rat lineages specifically for breast cancer research and to make these transgenic rats readily available to the breast cancer research community at a reasonable cost.

(2) To determine the most efficient technical procedures for the rapid generation of transgenic rat lineages on an outbred genetic background and on inbred genetic backgrounds appropriate for breast cancer research.

(3) To develop efficient procedures for rat embryo cryopreservation.

(4) To develop and maintain the necessary resources and establish procedures for ongoing data sharing and communication amongst transgenic rat laboratories and with breast cancer researchers.

To accomplish these specific aims, we developed a Statement of Work that incorporated aspects of all four specific aims into each of two chronological stages. Stage One dealt with the optimization of technologies for transgenic rat production and cryopreservation using outbred rat strains and Stage Two with optimization for transgenic rat production and embryo cryopreservation using inbred rat strains. The first stage of the Statement of Work, designed to cover years 1 and 2 of the grant period, included the following points:

(a) Using MMTV-*neu*^{WT} and MMTV-*neu*^{mut} as test DNAs, optimize variables in microinjection and embryo transfer in the outbred Sprague-Dawley background.

(b) Maintain a small breeding colony of the *neu* transgenic rats (6 lineages) for dissemination to other breast cancer researchers.

(c) Develop embryo cryopreservation for Sprague-Dawley rat embryos. Cryopreserve *neu* transgenic rat lineages.

(d) Solicit requests for DNAs from the breast cancer research community. Have advisory board choose DNAs, judged to be of the greatest potential value to breast cancer research, for microinjection during years 3 and 4.

(e) Develop and make available to transgenic rat and breast cancer research communities specialized information databases.

In the fourth year of the grant we continued efforts on Stage One and Two of the project. We have optimized cryopreservation procedures for another inbred strain of rat (Stage Two, Part D), cryopreserved multiple valuable transgenic rat strains (Stage One, Part C), continued to generate transgenic rats for breast cancer research on the Sprague-Dawley background (Stage Two, Part C), continued arrangements to generate transgenic rats for a breast cancer researcher outside the University of Wisconsin (Stage One, Part D), and initiated efforts to develop rat cloning as a means of providing a mechanism to generate rat knockouts (Stage Two, Parts B and C). The ensuing body of this report summarizes our work on the aims of this grant over the past year.

6. Body

6a. Statement of Work Point A (Specific Aim 1): Using MMTV-*neu*^{wt} and MMTV*neu*^{mut} as test DNAs optimize variables in microinjection and embryo transfer in outbred Sprague-Dawley background: Generation of transgenic rats carrying MMTV-*neu*^{wt} and MMTV-*neu*^{mut} DNAs.

An essential aim of this grant is to generate new valuable transgenic rat strains for breast cancer research. During the first years of this grant, we generated numerous transgenic rat lineages with several DNAs of interest to breast cancer research. These DNAs were as follows: First, MMTV-*neu*^{wt}, which consists of a mouse mammary tumor

virus long terminal repeat driving expression of the wild type neu protooncogene. The MMTV promoter sequences have been demonstrated to drive expression of linked genes to the mammary epithelium of transgenic mice (19-21) and, hence, would be expected to do so in the rat as well. The neu oncogene has been shown to be a frequently mutated gene in human breast cancers (26-28). Thus, a rat model where high levels of wild type neu would be expressed should be of value in evaluating the role of this protooncogene in breast cancer. Furthermore, such a rat model could be used in studies to evaluate the role of carcinogenic agents as cofactors in neu-associated breast cancers. The second DNA, called MT-*neu*^{mut} we chose to use is one where a mutated neu oncogene is fused to the mouse metallothionein promoter which is inducible by heavy metals such as zinc (22,23,25). The inducible approach was chosen to express the activated oncogene because of the worry that if expression of a mutated oncogene occurred too early in the life of the rat, stable rat lines would never be derived. The final DNA, called H*ras*-K*ras* consists of the transcriptional control regions of the H-*ras* gene fused to the coding sequences of the K-r*as* gene. Activated H-r*as*, but not K-*ras*, is frequently found in rat mammary carcinomas arising as a consequence of treatment with carcinogens (13,17). This transgene DNA is one of a series of transgenes designed to study the mechanisms whereby this differential activation occurs following carcinogen treatment. We had previously generated multiple transgenic rat lineages with the above DNAs. The results of studies with the MMTV-*neu*^{wt} lines in Dr. Michael Gould's laboratory in the Department of Human Oncology at University of Wisconsin indicated that generation of an additional line would be desirable. Thus, during the past year, we generated an additional line of MMTV-*neu*^{wt} transgenic rats. Although we had intended to maintain a small breeding colony of these rats for other investigators in the breast cancer research community to use, we have continued to delay these efforts until Dr. Gould's lab has completed the characterization of these transgenic rats. After Dr. Gould's laboratory has finished their assessment of the effects of deregulated neu expression on mammary carcinoma, we will reestablish a small breeding colony for the purpose of disseminating these animals to other interested investigators.

A goal listed for this grant was to solicit requests from the breast cancer research community at large for additional DNAs to use in the generation of transgenic rat models for breast cancer research (statement of work point d). We have arranged for a collaboration with Dr. Gail Sonenshein, Professor of Biochemistry at Boston University, who is studying the role of NF-kB/rel in mammary tumors. Her initial animal studies have used a mouse model, but she feels as though a rat model would be desirable. Given that her DNA construct is proven to express as expected in mouse, we anticipate success in generation valuable rat models for her work. Injections will begin with this DNA as soon as it is received from Dr. Sonenshein.

6b. Statement of Work Point A (Specific Aim 2): Using MMTV-*neu*^{wt} and MMTV*neu*^{mut} as test DNAs optimize variables in microinjection and embryo transfer in outbred Sprague-Dawley background: Optimize variables in microinjection and embryo transfer technique.

The second aim of our studies is to investigate ways to increase the efficiency and ease of transgenic rat production. During the first few years of this grant, we made significant progress towards evaluating the technical factors that influence the ultimate success in producing transgenic rats (refer to the progress report from year 1 for details on these experimental manipulations). After numerous studies, we concluded that we could not identify another variables that might affect microinjection efficiency that we could test. We were satisfied with the efficiencies we were obtaining. We concluded that this phase of the grant had been completed.

This page contains unpublished information.

6c. Statement of Work Point C (Specific Aim 3): Develop efficient methods for cryopreservation of Sprague-Dawley rat embryos.

As mentioned in background, we have established previously efficient methods for the cryopreservation of transgenic mouse embryos. It is clear that adaptation of this technology to the rat will be critical for the long term success of transgenic rat programs in our facility as well as world-wide. When we began our work to develop techniques for efficient cryopreservation of rat embryos, very little data were available in the literature; the only literature sources coming from Japanese groups who performed there studies exclusively with the Wistar strain of rats. (30, 31).

The freezing and thawing process requires that one be able to dehydrate the embryo with a cryoprotectant before freezing and then rehydrate the embryo after freezing without losing viability of the embryo. There are multiple variables that could affect embryo viability including the rate of freezing, the actual final temperature of freezing via controlled rate, the cryoprotectant used, the stage of embryo frozen. In previous years reports, we demonstrated that we had worked out the conditions necessary for successfully cryopreserving morulae from Sprague-Dawley rats. We additionally showed that we had determined that these procedures can be used to cryopreserve embryos from the inbred Wistar-Furth strain. Last year we reported that the efficient production of embryos for cryopreservation can be accomplished by superovulation regimens in which FSH and LH are administered to the female rat prior to mating. Last year we also reported that we had frozen and thawed hundreds of rat morulae with 90% viability which yielded approximately 35% live births when transferred into pseudopregnant recipients.

In the past year, we have determined that the superovulation and cryopreservation procedures that we have used for cryopreserving Sprague-Dawley and Wistar-Furth rats also are suitable for freezing morulae from the Copenhagen inbred strain. Approximately 90% of Copenhagen embryos were viable when thawed and 14% live births were recovered from embryos transferred into pseudopregnant recipients. While the percent of live births is low (about half that of Sprague-Dawley and Wistar-Furth), these results are derived from a very small sample size and therefore variables beyond our control may have affected recovery. While it is not certain that these exact conditions will be optimal for any strain, we believe that they are likely to be suitable for a wide variety of other rat strains and with limited experimentation could be optimized for other strains, if necessary.

In the Statement of Work, we indicated that we would cryopreserve various transgenic rat strains. To date we have cryopreserved one strain of MMTV-*neu*^{wt} transgenic rats, 3 strains of Hras-Kras transgenic rats and 5 strains of Hras-Hras transgenic rats for Dr. Gould's laboratory. Efforts to cryopreserve additional strains of transgenic rats are underway at present. These cryopreserved stocks provide a repository of these valuable strains that permits their "immortalization" without the huge time and cost investments that are required to maintain these animals alive. Additionally, cryopreservation protects these strains against disease, loss due to catastrophic events, and genetic drift. At any time in the future, these strains can be recovered for any interested researcher in the breast cancer field.

6d. Statement of Work Stage 2 Point B and C (Specific Aim 2) Using novel DNAs provided by breast cancer researchers and adapt transgenic rat technology to additional inbred rat backgrounds, complete optimization of microinjection and transfer technologies.

The transgenic technique of pronuclear microinjection allows one to add a function to the cell as a result of expression of a gene linked to an active promoter. Due to the

This page contains unpublished information.

technique, this type of experiment is limited, therefore, to introducing gain of function mutations. The gain of function could be the introduction of dominant acting mutations of cellular genes some of which may have a dominant-negative activity. However, it is often the case that once needs to determine the effect of recessive mutations on the biology of an organ such as the mammary gland. In mouse transgenic technology, gene targeting approaches that allow one to generate mutations directly in cellular genes are frequently utilized. These gene targeting technologies rely on the availability of embryonic stem ells which, when reintroduced into the mouse embryo, can contribute to all lineages of the mouse including the germline. Mutations are generated within the DNA in the embryonic stem cell and then these cells are microinjected into blastocysts to generate chimeric mice. Chimeric mice are then bred to establish lines of mice carrying the desired mutation and these mice are then interbred to produce mice with mutations in both alleles of a given gene. In theory, this same technology could be applied to the rat thereby opening up the possibilities for generating recessive mutations in cellular genes of the rat directly. This would be most advantageous for deriving animal models for diseases because the genetic basis for many diseases is recessive mutations in particular cellular genes. There has been a long standing effort to derive rat embryonic stem cells which retain the capacity to contribute to the germline when reintroduced into rat blastocysts. Unfortunately, to date, despite these many efforts, no one has derived such cells.

An alternative approach towards generating recessive mutations in the rat genome is suggested on the heels of the recent abilities to clone animals from somatic cells, as was the case for sheep named "Dolly" (32). In this approach one would generate the desired mutations by gene targeting in somatic cells that can be grown in culture. One cell clones carrying the desired mutations had been isolated and expanded, nuclei from these cells would be isolated and transferred into enucleated oocytes. The DNA from the somatic nucleus would then be deprogrammed in the environment of the oocyte and then, as the reconstituted embryo developed, be reprogrammed in keeping with developmental processes. Thus, a new individual is derived from the DNA of a somatic cell generating a identical copy, a clone.

During the past year, Dr. Warren in the laboratory has been investigating the feasibility of applying this technique to the rat, which we refer to as "rat cloning". The first requirement is that for the enucleation process, special epifluorescence equipment must be attached to the microinjection microscope. This capability allows one to visualize the metaphase plate in the oocyte which facilitates the complete removal of the pronucleus from the oocyte. Dr. Warren has purchased this equipment and become proficient at the enucleation step. The second requirement for this technique is the availability of suitable somatic cell lines in which to perform the gene targeting. Once targeted, these cells are the source of nuclei which are transferred into the enucleated oocyte. Although any somatic cell in theory can be used for this purpose, it is likely that cells of embryonic origin would be desirable because of the potential for more cell divisions in vitro before senescence. Embryonic fibroblasts are abundant, have good growth characteristics and are easy to establish in culture. Thus, Dr. Warren has established 6 lines of rat embryonic fibroblasts. The third requirement for this technique is that the nucleus from the somatic cell to be introduced into the enucleated oocyte. Two approaches are possible for this step, electrofusion or microinjection. The microinjection appears to be preferable because the embryo survival rate is 5 fold higher than the survival after electrofusion (33). Dr. Warren has purchased and set up the pieze-injector which is required for this microinjection manipulation and is currently becoming proficient at is use. The last requirement for this technique is that the recombinant oocytes be reintroduced into pseudopregnant recipients. However, prior to transfer, it is essential to culture the embryos to determine if they are viable. A question is how long the embryos can be cultured while maintaining their capacity to develop in utero. To this end, Dr. Warren has cultured various stages of rat embryos and

subsequently transferred them into the oviducts of pseudopregnant recipients to determine which combination of embryo stage and recipient stage is most supportive for embryonic development. Pregnancies were obtained from all stages of embryos used. This flexibility will permit the culturing of the recombined embryos through multiple cleavages before transfer. In sum, over the past year, Dr. Warren has set up many of the techniques that together are required for rat cloning. In the coming year, he will be putting all these steps together and optimizing conditions for rat cloning using stock embryonic fibroblasts. If he can achieve cloning, then it will be possible to move on to target genes in the embryonic fibroblast, then transfer them to occytes from which can be derived rats with targeted genes.

6e. Statement of Work Point E and F (Specific Aim 4): Develop and make available to transgenic rat and breast cancer research communities specialized information databases.

The ability to communicate easily, effectively and efficiently with others in transgenic research and breast cancer research is essential in today's rapidly moving scientific world. To this end, many find it useful to communicate using the Internet where bulletin boards are reaching their highest popularity. In the first year of this grant, we joined two "clubs" on the Internet: Rodent Research and Embryo Mail. Over the ensuing years, we continued to be involved in dialogue through these electronic means and continue to find these services effective vehicles for rapid and informal discussion with our colleagues.

An extensive, up to date, easy to use directory of transgenic animal researchers and breast cancer researchers who use animal models as their primary model system is necessary to facilitate communication between these large groups of investigators. A member of the University of Wisconsin Biotechnology Center's outreach team will be assisting us with this effort. This directory is being designed so that updates (additions, deletions) can be made easily. However, the outreach efforts of the Biotechnology Center have been reorganized and restricted recently. Thus, the individuals who were expected to carry out this function are longer available to us. Therefore, it has fallen on the hands of the scientists in the Transgenic Animal Facility to accomplish this task, in addition to their scientific efforts in the biological aspects of the project. Thus, progress is slow.

Within the transgenic animal research community, there are several databases, such as the TBASE database operated by the Johns Hopkins University, which aim to list most knockout mice and transgenic animals in existence. These groups have elected to make as their first priority accumulating all of the information on the knockout mice. Thus, the effort to accumulate transgenic mouse and especially transgenic rat data is secondary. Accomplishing our task will then enable us to devise a current listing of all transgenic rodent models for breast cancer research as well as all transgenic rat models. This database will be maintained and updated periodically so that at the end of the grant period, the current information can be transferred to larger transgenic database units in this country such as TBASE or similar databases.

7. Conclusions

During the past year, we have made excellent progress in achieving our goals which are to generate transgenic rat models for breast cancer research, improve the efficiency and ease with which transgenic rats are produced, to develop effective methods for cryopreservation of rat embryos and to develop the capacity for state of the art

This page contains unpublished information.

communication between the University of Wisconsin Transgenic Animal Facility and the transgenic rat and breast cancer research communities. We have generated additional lines of transgenic rat carrying the MMTV-*neu*^{wt} transgene. We have prepared to collaborate with a breast cancer researcher outside the University of Wisconsin. Because rat embryonic stem cells that contribute to the germline when microinjected into the embryo have still not been isolated, we have taken on the task of determining if rat cloning can be used as a means of generating rats carrying germline mutations in cellular genes. This is an essential technology to develop because the genetic basis of many diseases is recessive mutation in cellular genes and it is not possible to by dominant methods create the situation. We have progressed to the point where each of the required manipulations is likely to be feasible in our hands; necessary reagents have been generated and required equipment obtained. We have succeeded in developing an efficient straight forward rat embryo cryopreservation protocol that is applicable to multiple strains of rat and cryopreserved numerous transgenic rat lines. Lastly, we have begun to generate a current directory of transgenic rat researchers and breast cancer researchers using animal models in their work. This should help increase everyone's awareness of the state of the fields and facilitate communication between workers with overlapping research goals.

8. References

1. Medina, D. 1982. Mammary tumors. In *The mouse in biomedical research*, Vol. 4, (Foster, H.J. J.D. Small, and J.G. Fox, eds.) Academic Press, pp. 373-396.

2. Gould, M. 1993. Cellular and molecular aspects of multistage progression of mammary carcinogenesis in humans and rats. *Seminars in Cancer Biology* 4: 161-169.

3. Wang, B., W.S. Kennan, J. Yasukawa-Barnes, M.J. Lindstrom, and M.N. Gould. 1991. Frequent induction of mammary carcinomas following *neu* oncogene transfer into *in situ* mammary epithelial cells of susceptible and resistant rat strains. *Cancer Res.* **51**: 5649-5654.

4. Borg, A., F. Linell, I. Idvall, S, Johansson, H. Sigudsson, M. Ferno, and D. Killander. 1989. HER2/*neu* amplification and comedo type breast carcinoma. *Cancer Res.* **52**: 4102-4115.

5. Isaacs, J.T. 1987. Genetic control of resistance to chemically-induced mammary adenocarcinogenesis in the rat. *Cancer Res.* **46**: 3958-3963.

6. Gould, M.N. 1986. Inheritance and site of expression of genes controlling susceptibility to mammary cancer in an inbred rat model. *Cancer Res.* **5**: 62-81.

7. Haag, J.D., A. Newton, and M.N. Gould. 1992. Mammary carcinoma suppressor and susceptibility genes in the Wistar-Kyoto rat. *Carcinogenesis* **13:** 1933-1935.

8. Zhang, R., J.D. Haag, and M.N. Gould. 1990. Site of expression and biological function of the rat mammary carcinoma suppressor gene. *Carcinogenesis* **11**: 1765-1770.

9. Gould, M.N., B. Wang, and C.J. Moore. 1989. Modulation of mammary carcinogenesis by enhancer and suppressor genes. in *Genes and Signal Transduction in Multistage Carcinogenesis* (N.H. Colburn, ed.), Marcel Dekker, New York, pp. 19-38.

10. Coles, C., A. Condie, U. Chetty. C.M. Steel, H.J. Evans, and J. Prosser. 1992. p53 mutations in breast cancer. *Cancer Res.* 52: 5291-5298.

11. Malkin, D., F.P. Li, L.C. Strong, J.F. Fraumeni, C.E. Nelson, and D.H. Kim. 1990. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* **250**: 1233-1238.

12. Tsukamoto, A.S., R. Grosschedl, R.C. Guzman, T. Parslow, and H.E. Varmus. 1988. Expression of the *int*-1 gene in transgenic mice is associated with mammary gland hyperplasia and adenocarcinomas in male and female mice. *Cell* **55**: 619-625.

13. Andres, A.C., M.A. VanderValk, C.-A. Schoenenberger, F. Fluckinger, M. LeMeur, P. Gerlinger, and B. Groner. 1988. Ha-*ras* and c-*myc* oncogene expression interferes with morphological and functional differentiation of mammary epithelial cells in single and double transgenic mice. *Genes Dev.* **2**: 1486-1495.

14. Schoenenberger, C.-A., A.C. Andres, B. Groner, M. VanderValk, M. LeMeur, and P. Gerlinger. 1988. Targeted c-*myc* expression in mammary glands of transgenic mice induces mammary tumors with constitutive milk protein gene transcription. *Embo J.* **7**: 169-175.

15. Leder, A., P.K. Pattengale, A. Kuos, T.A. Stewart, and P. Leder. 1986. Consequences of widespread deregulation of the c-*myc* gene in transgenic mice: Multiple neoplasms and normal development. *Cell* **45**: 485-495.

16. Stewart, T.A., P.K. Pattengale, and P. Leder. 1984. Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/*myc* fusion genes. *Cell* **38**: 627-637.

17. Andres, A.C., O. Bchini, B. Schubaur, B. Dolder, M. LeMeur, and P. Gerlinger. 1991. H-*ras* induced transformation of mammary epithelium is favoured by increased oncogene expression or by inhibition of mammary regression. *Oncogene* **6**: 771-779.

18. Lucchini, F., M.G. Sacco, N. Hu, A. Villa, J. Brown, L. Cesano, L. Mangiarini, G. Rindi, S. Kindl, F. Sessa, P. Vezzoni, and L. Clerici. 1992. Early and multifocal tumors in breast, salivary, Harderian and epididymal tissues developed in MMTV-Neu transgenic mice. *Cancer Letters* **64**: 203-209.

19. Bouchard, L., L. Lamarre, P.J. Trembley, and P. Jolicoeur. 1989. Stochastic appearance of mammary tumors in transgenic mice carrying the MMTV/c-*neu*

20. Muller, W.J., E. Sinn, P.K. Pattengale, R. Wallace, and P. Leder. 1988. Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-*neu* oncogene. *Cell* **54**: 105-115.

21. Guy, C.T., M.A. Webster, M. Schaller, T.J. Parsons, R.D. Cardiff, and W.J. Muller. 1992. Expression of the *neu* protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. *Proc. Natl. Acad. Sci. USA* **89**: 10578-10582.

22. Tornell, J., L. Rymo, and O.G. Isaksson. 1991. Induction of mammary adenocarcinomas in metallothionein promoter-human growth hormone transgenic mice. *Int. J. Cancer* **49**: 114-117.

23. Jhappan, C., C. Stahle, R.N. Harkin, N. Fausto, G.H. Smith, and G.T. Merlino. 1990. TGFα overexpression in transgenic mice induces liver neoplasia and abnormal development of the mammary gland and pancreas. *Cell* **61**: 1137-1146.

24. Matsui, Y., S.A. Halter, J.T. Holt, B.L.M. Hogan, and R.J. Coffey. 1990. Development of mammary hyperplasia and neoplasia in MMTV-TGFα transgenic mice. *Cell* **61**: 1147-1155.

25. Sandgren, E.P., N.C. Luetteke, R.D. Palmiter, R.L. Brinster, and D.C. Lee. 1990. Overexpression of TGFα in transgenic mice: induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. *Cell* **61**: 1121-1135.

26. Clark, G.M. and W.L. McGuire. 1991. Follow-up study of HER-2/*neu* amplification in primary breast cancer. *Cancer Res.* **51**: 944-948.

27. Slamon, D. J., G.J. Clark, S.G. Wong, W.J. Levin, S.G. Stuart, J. Udove, A. Ullrich, W.L. McGuire. 1987. Human breast cancer: correlation of relapse and survival with amplification of the HER2/*neu* oncogene. *Science* **235**: 177-182.

28. King, C.R., Kraus, M.H., and S.A. Aaronson. 1985. Amplification of a novel v-*erb*B-related gene in human mammary carcinoma. *Science* **229**: 974-978.

29. Dragan, Y.P. and H.C. Pitot. 1992. The role of the stages of initiation and promotion in phenotypic diversity during hepatocarcinogenesis in rat. *Carcinogenesis* **13**: 739-750.

30. Miyoshi, K., H. Funahashi, K. Okuda, and K. Niwa. 1994. Development of rat one-cell embryos in a chemically defined medium: effects of glucose, phosphate and osmolarity. *J. Reprod. Fert.* **100**: 21-26.

31. Kasai, M., K. Niwa, and A. Iritani. 1982. Survival of rat embryos after freezing. *J. Reprod. Fert.* **66**: 367-370.

32. Wilmut, I., A.E. Schnieke, J. McWhir, A. Kind, and K.H.S. Campbell. 1997. Viable offspring derived from fetal and adult mammalian cells. *Nature* **385**: 810-813.

33. Wakayama, T., A.C.F Perry, M. Zuccottio, K.R. Johnson, and R. Yanagimachi. 1998. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature* **394**: 369-374.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y)

1 JUN 2001

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports. Request the limited distribution statement for reports on the enclosed list be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

Reports to be changed to "Approved for public release; distribution unlimited"

Grant Number	Accession Document Number
DAMD17-94-J-4147	ADB221256
DAMD17-93-C-3098	ADB231640
DAMD17-94-J-4203	ADB221482
DAMD17-94-J-4245	ADB219584
DAMD17-94-J-4245	ADB233368
DAMD17-94-J-4191	ADB259074
DAMD17-94-J-4191	ADB248915
DAMD17-94-J-4191	ADB235877
DAMD17-94-J-4191	ADB222463
DAMD17-94-J-4271	ADB219183
DAMD17-94-J-4271	ADB233330
DAMD17-94-J-4271	ADB246547
DAMD17-94-J-4271	ADB258564
DAMD17-94-J-4251	ADB225344
DAMD17-94-J-4251	ADB234439
DAMD17-94-J-4251	ADB248851
DAMD17-94-J-4251	ADB259028
DAMD17-94-J-4499	ADB221883
DAMD17-94-J-4499	ADB233109
DAMD17-94-J-4499	ADB247447
DAMD17-94-J-4499	ADB258779
DAMD17-94-J-4437	ADB258772
DAMD17-94-J-4437	ADB249591
DAMD17-94-J-4437	ADB233377
DAMD17-94-J-4437	ADB221789
DAMD17-96-1-6092	ADB231798
DAMD17-96-1-6092	ADB239339
DAMD17-96-1-6092	ADB253632
DAMD17-96-1-6092	ADB261420
DAMD17-95-C-5078	ADB232058
DAMD17-95-C-5078	ADB232057
DAMD17-95-C-5078	ADB242387
DAMD17-95-C-5078	ADB253038
DAMD17-95-C-5078	ADB261561
DAMD17-94-J-4433	ADB221274
DAMD17-94-J-4433	ADB236087
DAMD17-94-J-4433	ADB254499
DAMD17-94-J-4413	ADB232293
DAMD17-94-J-4413	ADB240900

Encl